DRAFT

Equine Influenza Vaccine, Killed Virus

List of Subjects

9 CFR Part 112

Animal biologics

9 CFR Part 113

Animal biologics

Accordingly, 9 CFR parts 112 and 113 would be amended as follows:

Part 112 - Packaging and Labeling

1. The authority citation for part 112 would continue to read as follows:

Authority: 21 U. S. C. 151 - 159; 7 CFR 2.22, 2.80, and 371.2(d).

2. Section 112.7 would be amended by adding new paragraph (n) to read as follows:

§112.7 Special Additional Requirements

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(n) In the case of biological products containing equine influenza virus, all labels shall specify the subtype(s) and strain(s) of the virus used in the product and the revaccination recommendation as determined from the results of duration of immunity studies acceptable to the Animal and Plant Health Inspection Service.

PART 113 - STANDARD REQUIREMENTS

3. The authority citation for part 113 would continue to read as follows:

Authority: 21 U.S.C. 151-159; 7 CFR 2.22, 2.80, and 371.2(d).

4. Section 113.217 would be added to read as set forth below.

§ 113.217 Equine Influenza Vaccine, Killed Virus

Equine Influenza Vaccine, Killed Virus, shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs. Only Master Seed which has been established as pure, safe,

and immunogenic may be used for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.200.

(b) The immunogenicity of vaccine prepared from the Master Seed in accordance with the Outline of Production must be established by the method prescribed in this paragraph or other method acceptable to the Animal and Plant Health Inspection Service. The vaccine used for this test must be at the highest passage from the Master Seed and at the minimum preinactivation titer provided in the Outline of Production. The test must establish that the vaccine when used as recommended on the label is capable of inducing an immune response that protects horses for at least 60 days following completion of the immunization regimen specified on the labeling.

(1) For at least one strain of each subtype of equine influenza virus contained in the vaccine, at least 15 susceptible horses of the minimum age recommended on the label shall be used as test animals. Horses are considered susceptible if the HI titer of individual serum samples taken from each animal is less than 1:10 using a constant virus, decreasing serum HI assay against 4 HA units of each strain of virus tested. The virus (antigen) may not be treated prior to the assay.

(2) At least 10 horses shall be vaccinated in accordance with the label recommendation, and at least 5 additional horses shall be held as unvaccinated controls. To demonstrate continued susceptibility, vaccinates must be negative for an anamnestic serologic response at 7 days after the first vaccination.

(3) Not less than 60 days after completion of the immunization regimen, the immunity of each of the vaccinates and the controls shall be challenged. At least 10 vaccinates, and 5 controls must be challenged with a representative strain of each equine influenza virus subtype present in the vaccine in a manner acceptable to APHIS, and observed each day for 7 days for clinical signs

of disease. Test animals must be bled immediately prior to challenge, and serum samples obtained for testing. If the controls are not seronegative at the time of challenge, the test is inconclusive and may be repeated.

(4) If a statistically significant ($p<0.05$) difference in clinical signs and temperature cannot be demonstrated between the vaccinates and controls using a scoring system acceptable to APHIS, the Master Seed is unsatisfactory.

(5) If the Master Seed immunogenicity test is satisfactory, other strains of equine influenza virus of the same subtype(s) may be added to the vaccine at any time by demonstrating that the added strain(s) elicits a serum HI titer either in horses or in guinea pigs that is equal to or greater than the titer elicited by the strain of the virus used in the challenge study. Provided, That:

(i) For each virus subtype claimed on the label for the product, the vaccine will at all times contain at least one strain of equine influenza virus whose immunogenicity has been determined in a host animal vaccination-challenge study.

(ii) Guinea pig HI titers may be used only if a satisfactory dose response relationship correlated to host animal protection and a mean relative potency value of the vaccine in guinea pigs based on a minimum of 3 replicate tests conducted at the time of the efficacy study has been established or can be shown.

(c) *Test requirements for release*. Each serial must meet the applicable general requirements prescribed in §113.200 and the special requirements for safety and potency provided in this section. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Safety test*. The vaccinates used in the potency test in paragraph (c)(2) of this section shall be observed each day during the post vaccination observation period. If unfavorable reactions occur which are attributable to the vaccine, the serial is unsatisfactory. If unfavorable

reactions occur which are not attributable to the vaccine, the test is inconclusive and may be repeated: Provided, That, if the test is not repeated, the serial is unsatisfactory.

(2) *Potency test*. Bulk or final container samples of completed product from each serial shall be tested for potency as provided in this paragraph. For each fraction of each subtype contained in the product--subtype A1 or subtype A2--the serological interpretations required in this test shall be made independently.

(i) At least 12 guinea pigs, each weighing between 300 and 500 grams, shall be used as test animals.

(ii) A dose of product equivalent to one-half the recommended horse dose shall be administered by the recommended horse route to at least 10 animals. A second dose shall be administered by the same route 14 to 21 days later. At least two animals shall be held as unvaccinated controls.

(iii) Fourteen to 21 days after the second vaccination, the animals shall be bled and serum samples obtained. The samples from each animal shall be tested in an HI assay consistent with that described in the following paragraph or by an alternative method acceptable to APHIS.

(iv) The serum samples shall be treated with kaolin and chicken red blood cells prior to initiation of the assay. A constant-virus, decreasing-serum HI assay against four hemagglutination units of each virus fraction shall be employed. The antigens may not be treated prior to performance of the assay.

(v) *Test interpretation.* If the controls for a given test fraction have not remained seronegative at the lowest test dilution (1:10), the test is inconclusive and may be repeated. If the geometric mean titer (GMT) of vaccinates in a valid test is less than the guinea pig GMT correlated with protection of horses against the applicable virus subtype, the serial is unsatisfactory unless the test is repeated. If the second test meets the requirements for validity

and the GMT of vaccinates from both tests is less than the guinea pig GMT correlated with protection of horses for that subtype, then the serial is unsatisfactory without further testing.

(d) If more than 60 days duration of immunity is to be claimed for any fraction, it may be shown by vaccinating at least 10 horses as recommended on the label and demonstrating an HI titer that is equal to or greater than the titer achieved in the Master Seed immunogenicity study for the period of time claimed. Labels must specify revaccination every 60 days if longer duration of immunity is not shown. Although not required, horses used to establish the duration of immunity beyond the required minimum of 60 days may also be challenged.

Done in Washington, DC, this _____ day of _____.